An evaluation of six marking methods for age-0 red drum, *Sciaenops ocellatus**

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Mark-recapture studies of fishes can reveal valuable information on movement, mortality, and growth rate (Parker et al., 1990). Despite the many successful methods that have been developed for adult fish, marking of small juvenile fishes is problematic (Chapman and Bevan, 1990). Age-0 fish are typically too small and delicate for many marking methods.

A few methods for marking age-0 fishes have been developed: coded wire microtags (Thrower and Smoker, 1984; Brodziak et al., 1992; Bumguardner et al., 1992); spray paint marking (Phinney et al., 1967; Pierson and Bayne, 1983); fluorescent staining (Hettler, 1984; Secor et al., 1991a; Szedlmayer and Able, 1992); and external plastic minitags (Floy Tag and Mfg. Co., Inc., Seattle, WA). All of these methods have advantages and disadvantages based on the attributes of each species. Thus, there is a need to test different marking methods on small size classes of different species to determine the most suitable methods.

Two marking methods have been reported for age-0 red drum, *Sciaenops ocellatus*: 1) spray paint marking,[1] and 2) coded wire microtags (Bumguardner et al., 1992). Fluorescent staining is another method that may be useful for age-0 *S. ocellatus* and has been successfully applied to juvenile red sea bream, *Pagrus major* (Tsukamoto et al., 1989), and to larval and juvenile striped bass, *Morone saxatilis* (Secor et al., 1991a). However, it is not possible from these studies to determine the most useful marking method for age-0 *S. ocellatus*. Hence, we examined mortality, mark retention, and growth in age-0 *S. ocellatus* that were marked by one of six different methods: 1) coded wire microtags, 2) external plastic minitags, 3) alizarin complexone, 4) oxytetracycline dihydrate [OTC], 5) red fluorescent spray paint, and 6) green fluorescent spray paint.

Materials and methods

We marked 614 cultured age-0 *S. ocellatus* (mean standard length [SL] ± standard deviation [SD]=67.4 ± 8.7 mm; range=48–95 mm SL) on 4–5 February 1993. Fish were anesthetized with tricane methanol sulfate (50 mg MS222/L seawater), weighed, measured, and randomly assigned to one of the following treatments: red fluorescent paint (red), green fluorescent paint (green), external plastic tags (plastic), binary coded wire microtags (wire), oxytetracycline-dihydrate (250 mg OTC/L 25 ppt seawater for 15 h), and alizarin-complexone (250 mg alizarin/L 25 ppt seawater for 15 h).

Wire tags were injected into the left epaxial muscle with a specially designed hypodermic needle (Northwest Marine Technology, Shaw Island, WA). The plastic tags (numbered disk: 0.5×3×7 mm) were attached posterior to the dorsal fin with elastic thread sewn through the left and right epaxial muscles. Red and green paints were applied at a pressure of 70 to 100 psi from a distance of 30 to 50 cm (Phinney et al., 1967). A total of 614 fish were marked and classified as follows: 100 OTC, 114 alizarin, 100 red, 100 green, 100 plastic, and 100 wire. All fish were held in a 7,570-L closed seawater system, and differentially marked fish were separated by flow through partitions. Ammonia, nitrite, and nitrate levels were controlled with an oyster shell biological filter (mean ± standard error [SE]: NH_3=0.018 ±0.003 ppm; NO_2=0.253 ± 0.047 ppm; NO_3=47.4 ± 1.9 ppm). Particulates were removed with a sand filter. Salinity was held constant by addition of artificial seasalts or freshwater (mean salinity ± SE=25.0 ± 0.2 ppt). Temperature was held constant with three 1,000-W heaters (mean temperature ± SE=20.8 ± 0.2°C).

Fish mortalities were counted and removed daily for 68 days. All fish were fed daily at 5% body weight per day with Zeigler salmon crumbles no. 2 pellet food (Zeigler Bros. Inc., Gardners, PA). At 25, 48, and 68 days, all fish from each treatment were anesthetized, weighed, measured, and food was adjusted for growth to maintain the daily 5% body weight ration. Red and green paint marks were verified with an ultraviolet light (paint was not visible under white light). Fish were considered marked if at least one granule of pigment was observed. Also, when fish were anesthetized and measured, we randomly sacrificed 20 fish from

* Contribution 8-944903 of the Alabama Agricultural Experiment Station, Auburn University, Mobile, Alabama 36615.

[1] McMichael, Robert H., Jr. Florida Department of Natural Resources, St. Petersburg, FL. Personal commun., 1992.

Manuscript accepted 29 August 1994.
Fishery Bulletin 93:191–195 (1995).

each treatment: from OTC and alizarin treatments for otolith mark examination, from wire treatments for wire removal, and from other treatments for future otolith analysis. All samples were preserved by freezing. In estimating percent survival, it was assumed that all fish that were sacrificed (alive and healthy at time of sample) would have survived the 68-d experimental period. The actual survival of sacrificed fish would be lower than 100%, but the difference in survival rate among treatments would be expected to increase if actual survival rates for all fish were known.

Whole sagittal otoliths were removed from OTC and alizarin treatments and viewed with an Olympus BH–2 compound microscope under 100-W ultraviolet light. When fluorescent marks were not visible on whole otoliths, they were sectioned and polished for increased resolution (Secor et al., 1991b; Szedlmayer and Able, 1992). Wire tags were located by making a sagittal incision of the epaxial muscle and examined under a Nikon stereo-microscope. If wire tags were not located by dissection, fish were X-rayed to locate tags.

We compared instantaneous mortality rates with analysis of covariance (ANCOVA) and percent tag retention with a randomized block analysis of variance (ANOVA) with day as blocks and marking method as the factor (Zar, 1984). We compared standard lengths (SL) and weights over marking method with ANOVA for each sample date. If significant differences ($P<0.05$) were detected, we compared the means (ANOVA) or slopes (mortality; ANCOVA) with Newman-Keuls range test (Zar, 1984).

Results

Instantaneous mortality rates (log percent survival=$-Zd + Y$) for OTC ($-Z$=0.0013, r^2=0.92), alizarin ($-Z$=0.0014, r^2=0.80), and wire ($-Z$=0.0016, r^2=0.78) marking were significantly less than those for plastic ($-Z$=0.0023, r^2=0.90), red ($-Z$=0.0025, r^2=0.96), and green treatments ($-Z$=0.0033, r^2=0.92; Fig.1). Survival curves showed similar patterns with higher mortality in the first 40 days; thereafter mortality was reduced (Fig. 1).

Percent mark retention of alizarin, OTC, and wire tags were significantly greater than those of other treatments. The highest mark retention was observed for OTC- and alizarin-marked fish (100%; Table 1). Wire-marked fish also showed high retention rates (85–100 %). Red-, green-, and plastic-marked fish showed significant declines in tag retention over the 68 days (Table 1).

Mean SL and weight showed no significant difference among treatments on day 1, 25, 48, and 68 (Tables 2 and 3). Growth rates were similar among all treatments: 1.0–1.1 mm SL/d and 0.5–0.6 g wet wt/d (Tables 2 and 3).

Discussion

Wire tags provided the best overall performance of the marking methods tested over this two-month

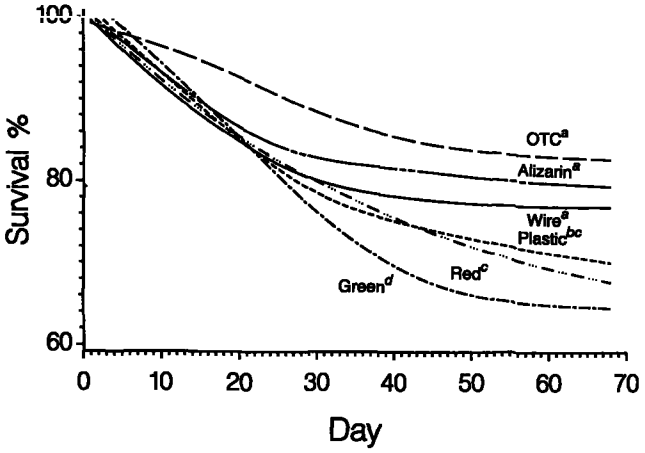

Figure 1

Percent survival of age-0 red drum, *Sciaenops ocellatus*, marked by six methods. Treatments with different letters were significantly different ($P<0.05$).

Table 1

Percent mark retention among sample days of age-0 red drum, *Sciaenops ocellatus*, marked by one of six methods: wire = coded wire microtag; plastic = external plastic tag; red = red fluorescent paint; green = green fluorescent paint; OTC = oxytetracycline dihydrate; and Ali = alizarin complexone. Numbers in parenthesis are sample sizes. Treatments with different letters are significantly different ($P<0.05$).

	Marking method					
Day	OTC[a]	Ali[a]	Wire[a]	Red[b]	Plastic[b]	Green[b]
25[a]	100.0(21)	100.0(20)	90.0(20)	80.5(82)	78.2(78)	42.3(78)
48[ab]	100.0(20)	100.0(20)	100.0(20)	55.8(52)	49.1(53)	39.1(46)
68[c]	100.0(19)	100.0(21)	85.0(20)	14.8(27)	24.1(29)	16.0(25)

study. Wire-marked fish showed low mortality and high tag retention compared with those marked by the plastic and paint methods. Also, individual fish are identifiable with wires which can be more useful than batch marking with OTC and alizarin. Of particular interest were the high tag-retention rates of wires (85–100 %). In past studies of wire tags injected in the cheek muscle of age-0 *S. ocellatus*, considerable tag loss was shown over the first 114 d: 67.3% tag retention after 24 h, 47% from 2 to 23 d, and 45%

Table 2

Mean standard lengths and growth rates of age-0 red drum, *Sciaenops ocellatus*, marked by one of six methods: wire = coded wire microtag; plastic = external plastic tag; red = red fluorescent paint; green = green fluorescent paint; OTC = oxytetracycline dihydrate; and Ali = alizarin complexone. Treatments were not significantly different ($P<0.05$). SL=standard length, SE=standard error, n=sample size.

		Mark method					
Day	Measure	Wire	Plastic	Red	Green	OTC	Ali
1	SL (mm)	65.9	66.9	66.8	67.1	68.9	67.6
	SE	0.8	0.9	0.9	0.9	0.9	0.7
	n	100	100	100	100	100	114
25	SL (mm)	90.5	91.6	88.8	90.5	90.7	91.6
	SE	1.0	1.1	1.1	1.1	1.1	1.0
	n	80	78	82	78	88	95
48	SL (mm)	116.7	117.9	112.5	118.1	117.5	114.0
	SE	1.3	1.7	2.0	1.6	1.6	1.4
	n	57	53	52	46	64	71
68	SL (mm)	131.1	134.7	134.4	134.3	128.7	129.1
	SE	2.2	2.9	2.0	2.3	1.8	2.1
	n	37	29	27	25	44	50
	Growth (mm/d)	1.1	1.1	1.1	1.1	1.0	1.0
	r^2	0.86	0.83	0.83	0.85	0.80	0.83

Table 3

Mean weights and standard error (SE) of age-0 red drum, *Sciaenops ocellatus*, marked by one of six methods: wire = coded wire microtag; plastic = external plastic tag; red = red fluorescent paint; green = green fluorescent paint; OTC = oxytetracycline dihydrate; and Ali = alizarin complexone. Treatments were not significantly different ($P<0.05$). WT=weight, n=sample sizes.

		Mark method					
Day	Measure	Wire	Plastic	Red	Green	OTC	Ali
1	WT (g)	4.9	5.2	5.2	5.2	5.7	5.2
	SE	0.2	0.2	0.2	0.2	0.2	0.2
	n	100	100	100	100	100	114
25	WT (g)	12.9	13.5	14.0	13.5	13.4	13.1
	SE	0.4	0.5	1.1	0.5	0.5	0.4
	n	80	78	82	78	88	95
48	WT (g)	30.1	29.5	27.9	28.2	27.8	27.2
	SE	1.1	1.3	1.4	1.1	1.1	1.0
	n	57	53	52	46	64	71
68	WT (g)	41.9	44.7	45.1	42.2	37.6	38.1
	SE	2.1	2.8	2.2	2.5	1.7	2.0
	n	37	29	27	25	44	50
	Growth (g/d)	0.6	0.6	0.6	0.6	0.5	0.5
	r^2	0.80	0.77	0.69	0.80	0.74	0.72

from 24 to 114 d (Bumguardner et al., 1992). A similar wire tag loss rate was observed in striped bass tagged horizontally in the cheek muscle (22.2–30.7% retention over the first 70 d; Dunning et al., 1990). However, Dunning et al., (1990) also reported that wire retention rates substantially increased when stripped bass were tagged in the snout (63–98.5%), nape (93.8–99.3%), or vertically in the cheek (82.7–87.0%) over the first 70 days. Also, Klar and Parker (1986) showed 99% tag retention after 90 days if wires were injected into the epaxial muscle of striped bass. Our results agree with the higher tag retention rates reported by Dunning et al., (1990) and Klar and Parker (1986); there was little indication of tag loss in the first days after marking. We suggest that higher wire retention resulted from mark location, because current wires were injected deep (4–5 mm) into the epaxial muscle and had little chance of expulsion.

Although wire tags showed the best overall performance compared with other tags, the method was the most labor intensive of all methods, because of the required dissection, removal, and reading of wires. If individual growth rates are needed and both personnel and budget are limiting, plastic tags may be useful. Plastic tags can be read directly without harm to the fish, but they also showed significant tag loss compared with other methods and should be limited to short experiments of no more than 25 days.

Paint marking methods showed greater mortalities and lower retention times compared with other methods but are useful in situations where fish can only be held for short periods (1–2 h, see Weinstien et al., 1984). However, paint marking methods should also be limited to short-term experiments because of significant mark loss after 25 days.

If fish movements or survival are the objectives and fish can be held for long (≈15 h) marking periods, then OTC or alizarin may be the best marking methods. Both methods showed higher tag retention (100%) and lower mortality rates compared with plastic minitags or paint methods. Other studies have shown long-term retention for these chemicals: for example, OTC marks in chum salmon, *Oncorhynchus keta* (Bilton, 1986) and alizarin marks in *P. major* (Tsukamoto et al., 1989) were both detected two years after marking. Another advantage of OTC and alizarin staining was that handling was minimal and probably caused the least amount of stress among all the marking methods, as reflected in the lower mortality rates. Therefore, these fluorescent stains would be most suitable for long-term studies where individual growth rates are not needed and for batch marking large numbers of fish, for example prior to release of hatchery reared fish (Tsukamoto et al., 1989; Secor et al., 1991a).

One difference in the present study was the use of oxytetracycline-dihydrate instead of oxytetracycline-hydrochloric acid (HCL), as used in other studies. (Hettler, 1984; Tsukamoto and Shima, 1990; Secor et al., 1991a). The dihydrate form of OTC was advantageous in that it did not cause a reduction in pH as observed with the HCL form. This difference may account for the low mortality observed after 15 hours in the OTC bath (i.e. no fish died during the 15-h marking period). One advantage of the alizarin stain over the OTC marker was the ease in detecting the fluorescent marks. When whole otoliths were examined only 2 of 91 alizarin otoliths needed further cutting and polishing to detect the stain, whereas 23 of 83 OTC otoliths needed sectioning before OTC marks were visible.

As indicated by growth rates, fish acclimated well to the closed seawater system. Although we did not have a control group of fish, growth rates in the present study (1.0–1.1 mm SL/d) were similar to or greater than previous studies of unmarked *S. ocellatus* of similar sizes and temperatures as the current study: 0.8 mm/d from Tampa Bay (Peters and McMichael, 1987), 0.8 mm/d from Charleston Harbor (Daniel, 1988), and 1.0 mm/d for reared fish in Texas (Colura et al., 1990). Also, we were not attempting to compare growth rates of marked fish with those of wild populations or those of unmarked laboratory fish but rather to determine the most useful tag of the methods tested.

Thus, we recommend wire tags when individual marks are needed because of lower mortality and higher mark retention compared with those from plastic minitags. We recommend alizarin when batch-marking methods are needed and individual growth rates are not critical, because of lower mortality and higher retention compared with those from paint methods and because of ease of detecting mark compared with OTC marking.

Acknowledgments

We thank J. Lindstrom and J. Mang for help in rearing and sampling *S. ocellatus*. We thank L. Collins for review of an early draft. This research was funded through a Saltonstall-Kennedy grant USDC-NA27FD0063-01, National Marine Fisheries Service, National Oceanic and Atmospheric Administration.

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